Figure 1. The Naegleria agent causes the rapid disappearance of thiamin from the growth medium of rat glioma C6 cells.

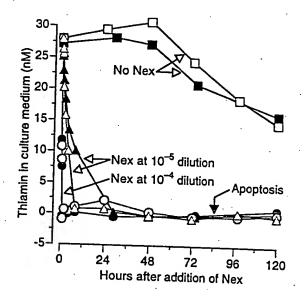


Figure 2. The addition of thiamin can reverse progress of rat glioma C6 cells toward apoptosis induced by the *Naegleria* agent. Thiamin added at 12 hour intervals reversed progress until death of the cells had already occurred on day 4.5.

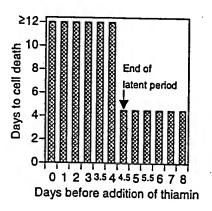


Figure 3. An SDS-polyacrylamide gel stained for thiaminase and for protein. The *Naegleria* agent was purified through preparative isoelectric focusing (see Example 3).

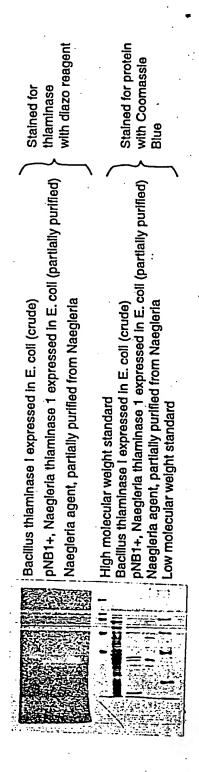


Figure 4. The complete DNA sequence of the coding region of *Naegleria* gene TTK (including the TAA stop codon). The segment of the gene that encodes thiaminase I is underlined.

<u>ATTGATCAATTTGATCAAGGATGTTTTGCCAACTCAGGTTTCCGGATATAATATCGAATATACCGAATTTGATTGTTACA</u> GTCAAGAGTTTGGATGAATCATTGGTTCGTGGTGTTACTGGTGATTTGCATAGTTTTGTTTCCTCAAGTGCCTCTGT TGGTTCCGTTTATGGTTTCCCACAATACTTGTGCTCAAACTTTTTATTGTCCTCACCAAATGGTACTCAACAAGCATCTT CCCTTTTAGAATTGGCTCAAAAGGTTGGTTATGAACAAATTGTTTATCCAGATGTTGCCTCTTCTAGTTCTTTCACAGTT TTCGGATTGTATCAACAATTACTCCAATCATCATCATCAGCTGCAGTTGATATCAAGGCCTCTGATCTTCCACAATCTGG TGACCAAGTCAACAAGGATATCACTCAAAAATATAGAACCATTTTGGATTCAACAGTTGTTGCCTCTCAAAGAGAATATA TTAACTCTGTAAAGCAAGGTAAACCAATTTCAAACTACTATGTCGGATATAGTGAAAGTATGTGGAAATTAAGGATATC ATCAGAGATCAACAATACAATGTTCAACTCATTGGTACCTCTGATAAGCCATACGTTTATACTGATGTTTTGGCTTTGAA ${f T}{f T}{f C}{f A}{f A}{f T}{f T}{f G}{f A}{f A}{f A}{f G}{f G}{f T}{f G}{f C}{f T}{f G}{f A}{f G}{f A}{f T}{f A}{f C}{f A}{f A}{f A}{f T}{f T}{f A}{f C}{f T}{f A}{f A}{f C}{f T}{f A}{f C}{f C}{f$ TGTTGGGTCTCGGATTAACTCTCCCAGCCAACAAGAATGGTATTGCTCATTTGGCTAAATCATCAAACTTTTATGCTCAA **TTGAGCCAACAATTCGATGCCAAGGAAAGTGAAGTTAGAGTTTTGAGATGTGTTGACTTTGCTAACAAGGAAGTTAAGAA** ${f TTGTGCTGGTGTCTTGAGACCATTCCTT}$ CAACATATTGCTGTTGCTACTTTGCGTTGTTTGACTGCTGACACTGTCGAAA AGGCTAAGAGTGGTCACCCTGGTATGCCAATTGGTATGTCACCAATTGCCTATGTTTTGTGGAAGTTCTTCAAATCA ${ t TCTAAGGATGATGTCAATTGGTTGAACAGAGATAGATTTGTTTTGAGTAATGGTCACGGTTGTACATTGCTTTATGCCAT$ GTTGCACCTCACTGATTGTAACTTGAGTTTGGATGATCTCAAGAATTTCAGAAGTTTGCATTCCAAGACTCCTGGTCACC CAGAATATGGTCACACTGAAGGTGTTGATGCTACTGGTCCATTGGGTCAAGGTGTTTGTAATGCTATTGGTATGGCT CTCTCTGAAGCTCACTTGGCTGCTCGTTTCAATAAGGATGGACAAAATATCTTTGATCACCACACCTATGTTTTCCTTGG TGATGGTTGTTTGATGGAACGTGTTGCTATGGAAGGTCTCTCATTTGCTGGTCACCAAAAGTTGAACAAGTTGATTGTTT TCTATGATGACAATAGTATTACTATTGATGGTAAGACTGAATTGACCTTTACTCAAAATACTCCAGAAGTCATGAGAGGT TGTTACTGACAAGCCAATCATGATCGTTTGTAAGACTACAATTGGTTATTCCTCAAAGGTTCAAGGTACTGCTAAGGTTC ${\tt ACGGTTCTCCATTGGGTGCTGATGGATTGAAGAATTTGAAGGAAACTTGTGGTTTCACTGGTAATGATTTCTTCCATGTT}$ CCAGAAATTGTCAGAAAGGACTTTGCTACTGTCATTAATAGAAATAGTGAAAAGCTCTCTCAATGGAAGCAAGTTAAATC TGCCTATGATACCACTCATGCTACTGAATCCCAACTCCTCCAAAGAATGATTAATCACGAATTGGAAGGTGATGTTATGG AAAAGTTGCCAAAATACCTCGAACAAAAGAAGATTGCTACCAGATCTACATCTCAACAAGTTTTGAATGCCATCTATCCA TTGTCTGGTCTTCCAAATATTTATGTTTTCACTCATGACAGTATTGGTCTTGGTCAAGATGGTCCAACTCACCAACCTGT TGAAGTTTTACCAATGTTGATAGCCATTCCAAATCACATTGTTTTCAGACCTGCTGATGGTAGAGAAACCAGTGGTGCTT GATATTTCAAAGGTTGCTTTGGGTGCCTATGTTATCCAAGGTGATGCTACTCCTGATGTTGTCCTTGTTGGTACTGGTTC TGAAGTTTCCCTCATGGTTGAAGCTGCTGAAAAGTTGAAGGCTAACCTTAAGGTTAACGTTGTTTCCATGCCAAGTTGGG ${\tt AATTGTTTGTTCGTCAATCAGAAGAATACAGGAAGACTGTCTTCCCAGATGGTATTCCAGTTGTCAGTGCCGAAGCTTCA}$ CAAGACTCAGCTTGTCTCTTGTTGGTGAAGAACTCTAA

Figure 5. The amino acid sequence encoded by the entire Naegleria TTK gene

MSTQPKTLTVGLFPYLPSWNENGNEVKLINLIKDVLPTQVSGYNIEYTEFDCYSDASLQSLPDVFSTDSIFLPYLVSLGG VKSLDESLVRGVTGDLHSFVSSSASVNGSVYGFPQYLCSNFLLSSPNGTQQASSLLELAQKVGYEQIVYPDVASSSSFTV FGLYQQLLQSSSSAAVDIKASDLPQSGDQVNKDITQKYRTILDSTVVASQREYINSVKQGKPISNYYVGYSESMCEIKDI IRDQQYNVQLIGTSDKPYVYTDVLALNSNLCDEKQKVAVEVIKNLLTNTLVLDLLGLGLTLPANKNGIAHLAKSSNFYAQ LSQQFDAKESEVRVLRCVDFANKEVKNCAGVLRPFLQHIAVATLRCLTADTVEKAKSGHPGMPIGMSPIAYVLWKFFFKS SKDDVNWLNRDRFVLSNGHGCTLLYAMLHLTDCNLSLDDLKNFRSLHSKTPGHPEYGHTEGVDATTGPLGQGVCNAIGMA LSEAHLAARFNKDGQNIFDHHTYVFLGDGCLMERVAMEGLSFAGHQKLNKLIVFYDDNSITIDGKTELTFTQNTPEVMRG FGWHVIVVDKADNDLVGIKEAILEAHTVTDKPIMIVCKTTIGYSSKVQGTAKVHGSPLGADGLKNLKETCGFTGNDFFHV PEIVRKDFATVINRNSEKLSQWKQVKSAYDTTHATESQLLQRMINHELEGDVMEKLPKYLEQKKIATRSTSQQVLNAIYP LIPSLVGGSADLTPSNLTDVTGCQDFQPNNRVGRYIRFGVREHAMVAIANGILYHGVLRTYVGTFLNFASYALGAIRLSA LSGLPNIYVFTHDSIGLGQDGPTHQPVEVLPMLIAIPNHIVFRPADGRETSGAYLWAVQSKKTPSSMILSRQDLPQLTGT DISKVALGAYVIQGDATPDVVLVGTGSEVSLMVEAAEKLKANLKVNVVSMPSWELFVRQSEEYRKTVFPDGIPVVSAEAS STFGWTSFAHYAVGMTTFGASAAAEEVYKLLKITSDNVAEKATKLVTKYGKQAPRLSLSLVGEEL

Figure 6. The DNA sequence of the 1068 bp *Naegleria* thiaminase I segment, as cloned in pNB1+ and expressed from the 3414 base genomic sequence of gene TTK.

Figure 7. The encoded amino acid sequence of the expressed *Naegleria* thiaminase I gene, along with its codons, and with residues numbered (nucleotide/amino acid).

ATG TCC ACT CAA CCA AAG ACA CTC ACT GTT GGT CTC TTC CCA TAT CTT CCT TCT TGG AAT Met ser thr gln pro lys thr leu thr val gly leu phe pro tyr leu pro ser trp asn GAA AAT GGC AAC GAA GTT AAA TTG ATC AAT TTG ATC AAG GAT GTT TTG CCA ACT CAG GTT glu asn gly asn glu val lys leu ile asn leu ile lys asp val leu pro thr gln val 151/51 TCC GGA TAT AAT ATC GAA TAT ACC GAA TTT GAT TGT TAC AGT GAT GCT AGT CTT CAA AGT ser gly tyr asn ile glu tyr thr glu phe asp cys tyr ser asp ala ser leu gln ser 211/71 CTT CCA GAT GTT TTC TCA ACT GAT AGC ATT TTC CTT CCA TAT CTT GTT TCT TTG GGT GGT leu pro asp val phe ser thr asp ser ile phe leu pro tyr leu val ser leu gly gly 271/91 GTC AAG AGT TTG GAT GAA TCA TTG GTT CGT GGT GTT ACT GGT GAT TTG CAT AGT TTT GTT val lys ser leu asp glu ser leu val arg gly val thr gly asp leu his ser phe val 301/101 TCC TCA AGT GCC TCT GTC AAT GGT TCC GTT TAT GGT TTC CCA CAA TAC TTG TGC TCA AAC ser ser ala ser val asm gly ser val tyr gly phe pro glm tyr leu cys ser asm TTT TTA TTG TCC TCA CCA AAT GGT ACT CAA CAA GCA TCT TCC CTT TTA GAA TTG GCT CAA phe leu leu ser ser pro asm gly thr glm glm ala ser ser leu leu glu leu ala glm AAG GTT GGT TAT GAA CAA ATT GTT TAT CCA GAT GTT GCC TCT TCT AGT TCT TTC ACA GTT lys val gly tyr glu gln ile val tyr pro asp val ala ser ser ser phe thr val 511/171 phe gly leu tyr gln gln leu leu gln ser ser ser ala ala val asp ile lys ala 541/181 571/191 TCT GAT CTT CCA CAA TCT GGT GAC CAA GTC AAC AAG GAT ATC ACT CAA AAA TAT AGA ACC ser asp leu pro gln ser gly asp gln val asn lys asp ile thr gln lys tyr arg thr 601/201 631/211 ATT TTG GAT TCA ACA GTT GTT GCC TCT CAA AGA GAA TAT ATT AAC TCT GTA AAG CAA GGT ile leu asp ser thr val val ala ser gln arg glu tyr ile asn ser val lys gln gly AAA CCA ATT TCA AAC TAC TAT GTC GGA TAT AGT GAA AGT ATG TGT GAA ATT AAG GAT ATC lys pro ile ser asn tyr tyr val gly tyr ser glu ser met cys glu ile lys asp ile 751/251 ATC AGA GAT CAA CAA TAC AAT GTT CAA CTC ATT GGT ACC TCT GAT AAG CCA TAC GTT TAT ile arg asp gln gln tyr asn val gln leu ile gly thr ser asp lys pro tyr val tyr 781/261 811/271 ACT GAT GTT TTG GCT TTG AAT TCC AAT TTG TGT GAT GAA AAG CAA AAG GTT GCT GTT GAA thr asp val leu ala leu asm ser asm leu cys asp glu lys glm lys val ala val glu GTT ATC AAG AAT TTA TTG ACT AAT ACT TTA GTT TTG GAC TTG TTG GGT CTC GGA TTA ACT val ile lys asn leu leu thr asn thr leu val leu asp leu leu gly leu gly leu thr l 931/311 CTC CCA GCC AAC AAG AAT GGT ATT GCT CAT TTG GCT AAA TCA TCA AAC TTT TAT GCT CAA leu pro ala asn lys asn gly ile ala his leu ala lys ser ser asn phe tyr ala gln 991/331 TTG AGC CAA CAA TTC GAT GCC AAG GAA AGT GAA GTT AGA GTT TTG AGA TGT GTT GAC TTT leu ser gln gln phe asp ala lys glu ser glu val arg val leu arg cys val asp phe 1021/341 1051/351 GCT AAC AAG GAA GTT AAG AAT TGT GCT GGT GTC TTG AGA CCA TTC CTT ala asn lys glu val lys asn cys ala gly val leu arg pro phe leu

Figure 8. Comparison of the amino acid sequence of *Naegleria* thiaminase 1 (N40KAT) aligned with the sequences of *Bacillus* thiaminase (BSTP) and segments of the transketolases of six organisms. The numbering system indicated here is for the yeast transketolase.

Abbrev.	Genbank	Enzyme and Organism
ScTKT1ct -	P23254	Transketolase of Saccharomyces cerevisiae
CpTKT7ct -	042677	Transketolase of Craterostigma plantagineum
EcTKT2ct -	P33570	Transketolase of Escherichia coli
BsTKTct -	P45694	Transketolase of Bacillus subtilis
MgTKT -	P47312	Transketolase of Mycoplasma genitalium
MjPTK1 -	Q58092	Transketolase of Methanococcus jannaschii
BSTP -	P45741	Thiaminase I precursor from Bacillus thiaminolyticus
N40KAT -	>	Thiaminase I of Naegleria gruberi, aa 1-356
ScTKT1ct		LKSKFGFNPDKSFVVPQEVYD-HYQKTILKPGVEANNKWNKLFSEYQKKFP 56
CpTKT7ct		ATRKNLGW-PYEPFHVPDDVKK-HWSRHIAE-GAALESAWNAKFAEFQKKFP
EcTKT2ct		LARQKLGW-HHPPFEIPKEIYHAWDAREK-GEKAQQSWNEKFAAYKKAHP
BsTKTct		TKEAYAWTYEEDFYVPSEVYE-HFAVAVKESGEKKEQEWNAQFAKYKEVYP
MgTKT	EVDFQI	FEKRTNT-NFNFFNYPDSIYH-WFKQTVIERQKQIKEDYNNLLISLKDK-P
MjPTK1		
BsTP		(KPLMVMLALLLVVVSPAGAGAAHSDASSDITLKVAIYPYVPDPARFQAAVL
N40KAT	MSTQP	-KTLTVGLFPYLPSWNENGNEVKLINLIKDVLPT
Cemumi		
ScTKT1ct		RLSGQLPANWESKLPTYTAKDSAVATRKLSETVLEDVYNQLPELIGGS 112
CpTKT7ct	EEAADLKS.	ITGELPTNWESIFPTYTPENPGLPTRTLSHQILNGLGDVLPGLLGGS
EcTKT2ct	QLAEEFTRI	RMSGGLPKDWEKTTQKYINELQANPAKIATRKASQNTLNAYGPMLPELLGGS
BSTKTct	ELAEQLEL	AIKGELPKDWDQEVPVYE-KGSSLASRASSGEVLNGLAKKIPFFVGGS
MgTKT	-LFKKFTN	WIDSDFQALYLNQLDEKKVAKKDSATRNYLKDFLNQINNPNSNLYCLN
MjPTK1		MVKLSGVYKGMRKGYGETLIELGKKYENLVVLD
BsTP		GVKLEF-TDWDSYSADPPDDLDVFVLDSIFLSHFVDAGYLLP-FGSQD
N40KAT	QVS0	GYNIEY-TEFDCYSDASLQSLPDVFSTDSIFLPYLVSLGGVKSLDESLV
		:
ScTKT1ct	A DY MDONT	
CpTKT7ct	ADLTPSNL.	TRWKEALDFQPPSSGSGNYSGRYIRYGIREHAMGAIMNGISAFGANYKPYGG 172
EcTKT2ct	ADLTLSNM	AFLKNSGDFQKKSPGERNVKFGAREHAMGSICNGLALHSPGLLPYCA
BsTKTct	ADLAPSNL.	TIWKGSVSLKEDPAGNYIHYGVREFGMTAIANGIAHHG-GFVPYTA
	ADLAGSNK:	TTIKNAGDFTAVDYSGKNFWFGVREFAMGAALNGMALHG-GLRVFGG
MgTKT MjPTK1	ADVSKSC	CFIKIGDDNLHENPCSRNIQIGIREFAMATIMNGMALHG-GIKVMGG
BsTP	ADLSGS	TQTAMFAKEFPERFFNAGVAEQNMIGMAAGLATTGKIVFAS
N40KAT		PFALQGAKRNGEVYGLPQILCTNLLFYRKGDLKIGQVDNIYELYKKIG
NAOKAI		FVSSSASVNGSVYGFPQYL <u>C</u> SNFLLSSPNGTQQAS-SLLELAQ
	. : .	
		Catalytic Cys
ScTKT1ct	ጥድ፣.እምነ <i>ነ</i> ር _1	(AAGAVR-LSALSGHPVIWVATHDSIGV-GEDG-PTHQPIETLAHF 222
CpTKT7ct		MRAAMR-ISALSGHPVIWVATHDSIGV-GEDG-PTHQPIETLAHF 222 MRAAMR-ISALSKARVLYIMTHDSIGL-GEDG-PTHQPVEHLASF
EcTKT2ct		
BsTKTct		/ARNAAR-MAALMKARQIMVYTHDSIGL-GEDG-PTHQAVEQLASL /LRPAIR-LAALMGLPVTYVFTHDSIAV-GEDG-PTHEPVEQLASL
MgTKT		KERPATR-LAALMGLPVTYVFTHDSTAV-GEDG-PTHEPVEQLASL KSKPATR-LGALMNLPVFYVYTHDSYQV-GGDG-PTHQPYDQLPML
MiPTK1	SESMEN SCI	RAWEIIRNLVAYPKLNVKIVATHAGITV-GEDG-ASHQMCEDIAIM
BsTP	TCHCECTE	PPQNKGLLINMAGGTTKASMYLEALIDVTGQYTEYDLLPPLDPLNDKVIRGL
N40KAT	KACAEVIL	PPUNGSBITNEAGGTTRASMTBEABIDVTGQYTEYDBLPPBDPLNDKVIRGB PDVASSSSFTVFGLYQQLLQSSSSAAVDIKASDLPQSGD-QVNK
	KAGIEQIV:	
•	•	Power and a second control of the second con

Peptide A -> ASDLPQSGD-QVNK

Figure 8, continued.

SCTKTICE CPTKT7CE ECTKT2CE BSTKTCE MGTKT MjPTK1 BSTP N40KAT	RSLPNIQVWRPADGN-EVSAAYKNSLESKHTPSIIALSRQNLPQLEGSSIESASKGG RAMPNILTLRPADGN-ETAGAYRAAVQNGERPSILVLARQKLPQLPGTSIEGVSKGG RLTPNFSTWRPCDQV-EAAVGWKLAVERHNGPTALILSRQNLAQVERTPD-QVKEIARGG RAMPNLSLIRPADGN-ETAAAWKLAVQSTDHPTALVLTRQNLPTIDQTSEEALAGVEKGA RAIENVCVFRPCDEK-ETCAGFNYGLLSQDQTTVLVLTRQPLKSIDNTDSLKTL-KGG RAIPNMVVIAPTDYY-HTKNVIRTIAEYKG-PVYVRMPRRDTEIIYENEEEATFEIGKGK RLLINMAGEKPSQYVPEDGDAYVRASWFAQ-GSGRAFIGYSESMMRMGDYAEQVRFK DITQKYRTILDSTVV-ASQREYINSVKQGK-PISNYYVGYSESMCEIKDIIRDOOYN	
N40KAT	DITQKYRTILDSTVV-ASQREYINSVKQGK-PISNYYVGYSESMCEIKDIIRDQQYN	

Peptide B -> TILDSTVV-ASQR

ScTKT1ct CpTKT7ct EcTKT2ct BsTKTct MgTKT MjPTK1 BsTP	YVLQDVANPDIILVATGSEVSLSVEAAKTLAAKNIKARVVSLPDFFTFDKQPLE-332 YVISDNSRGGNSKPDVILIGTGSELEIAARAGDELRKEGKKVRVVSLVCWELFAEQSEK- YVLKDSGGKPDIILIATGSEMEITLQAAEKLAGEGRNVRVVSLPSTDIFDAQDEE- YVVSKSKNE-TPDALLIASGSEVGLAIEAQAELAKENIDVSVVSMPSMDRFEKQSDE- YILLDRKQPDLIIAASGSEVQLAIEFEKVLTKQNVKVRILSVPNITLLLKQDEK- ILVDGEDLTIIATGEEVPEALRAGEILKENGISAEIVEMATIKPIDEEIIKK PISSSAGQDIPLFYSDVVSVNSKTAHPELAKKLANVMASADTVEQALRPQADGQ
N40KAT	PISSSAGQDIPLFYSDVVSVNSKTAHPELAKKLANVMASADTVEQALRPQADGQ VQLIGTSDKPYVYTDVLALNSNLCDEKQKVAVEVIKNLLTNTLVLDLLG- * : : : : : : : : : : : : : : : : : :

ScTKT1ct YRLSVLPDNVPI-MSVEVLATTCWGKYAH-----QSFGIDRFGASGKAPEVFKFFGFTP 385 CpTKT7ct YRETVLPSGVTARVSVEAGSTFGWERFIGP-KG--KAVGIDRFGASAPAERLFKEFGITV EcTKT2ct YRESVLPSNVAARVAVEAGIADYWYKYVGL-KG--AIVGMTGYGESAPADKLFPFFGFTA BsTKTct YKNEVLPADVKKRLAIEMGSSFGWGKYTGL-EG--DVLGIDRFGASAPGETIINEYGFSV MgTKT YLKSLFDANSSL-ITIEASSSYEWFCFKKY-VKNHAHLGAFSFGESDDGDKVYQQKGFNL MjPTK1 ${\tt SKDFVVTVEDHSIIGGLGGAVAEVIASNGLNKKLLRIGINDVFGRSGKADELLKYYGLDG}$ **BsTP** YPQYLLPARHQV-YEALMQDYPIYSELAQIVNK--PSNRVFRLGPEVRT-WLKDAKQVLP N40KAT -LGLTLPANKNG-IAHLAKSSNFYAQLSQ-----QFDAKESEVRVLRCVDFANKEV

Peptide C -> SSNFYAQLSQ-----QFDAK

ScTKT1ct	EGVAERAQKTIAFYKGDKLISPLKKAF	412
CpTKT7ct	EAVVA-AAKEIC	
EcTKT2ct	ENIVAKAHKVLGVKGA	
BsTKTct	PNVVNRVKALINK	
MgTKT	ERLMKIFTSLRN	
MjPTK1	ESIAKRIMEEMKKE	
BsTP	EALGLTDVSSLAS	
N40KAT	KNCAGVLRPFL	